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Silymarin Extraction from Milk Thistle Using Hot Water

Lijun Duan,[1] Danielle Julie Carrier,[2] and Edgar C. Clausen*,[1]

[1]Department of Chemical Engineering, University of Arkansas, 3202 Bell Engineering Center, Fayetteville, AR 72701, E-mail: eclause@engr.uark.edu; and [2]Department of Biological and Agricultural Engineering, University of Arkansas, 203 Engineering Hall, Fayetteville, AR 72701

Abstract

Hot water is attracting attention as an extraction solvent in the recovery of compounds from plant material as the search for milder and "greener" solvents intensifies. The use of hot water as an extraction solvent for milk thistle at temperatures above 100°C was explored. The maximum extraction yield of each of the silymarin compounds and taxifolin did not increase with temperature, most likely because significant compound degradation occurred. However, the time required for the yields of the compounds to reach their maxima was reduced from 200 to 55 min when the extraction temperature was increased from 100 to 140°C. Severe degradation of unprotected (plant matrix not present) silymarin compounds was observed and first-order degradation kinetics were obtained at 140°C.

Index Entries: Milk thistle; extraction; silymarin; hot water; silybinin; taxifolin; silychristin.

Introduction

Milk thistle (*Silybum marianum*) is an annual or biennial plant native to the Mediterranean that now grows wild throughout Europe, America, and Australia. The active ingredients in milk thistle seeds are the dihydroquercitin taxifolin, and the flavanolignans silybinin (present as silybinin A and B), isosilybinin, silydianin, and silychristin. The structures of these compounds are shown in Fig. 1. The flavanolignans, also referred to as silymarin, are found throughout the entire milk thistle plant but are concentrated in the fruits and seeds *(1)*.

*Author to whom all correspondence and reprint requests should be addressed.

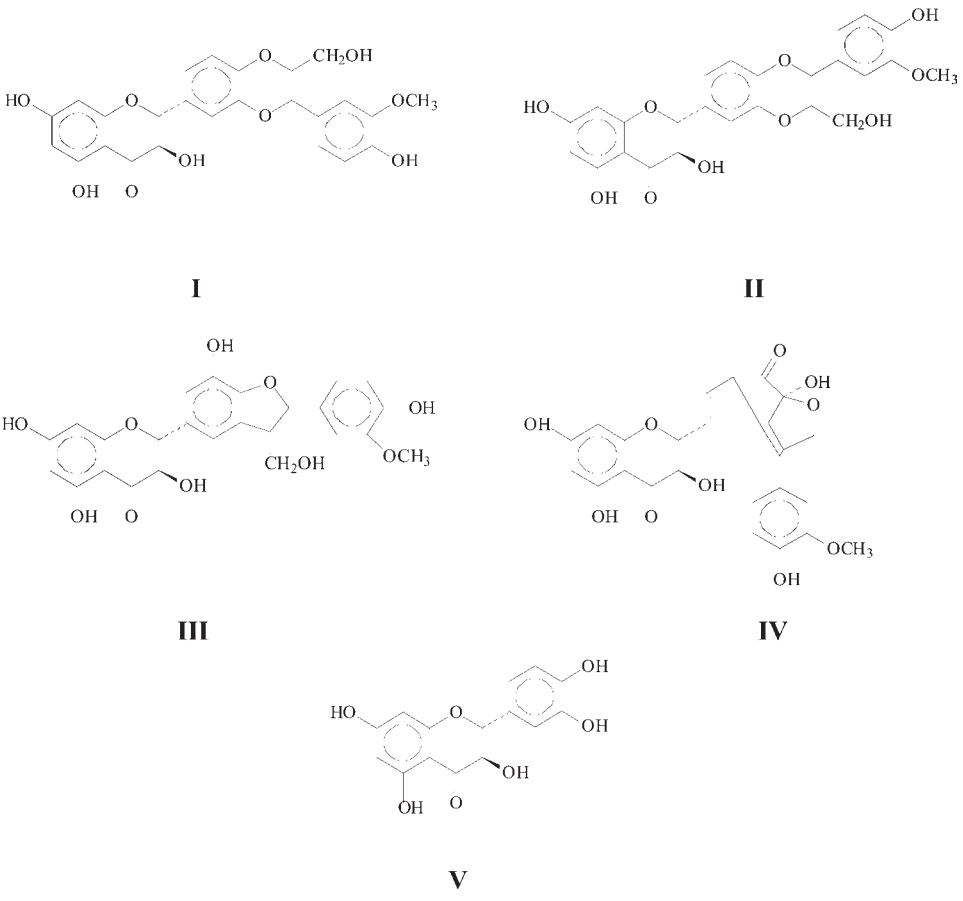

Fig. 1. Structures of dihydroflavanol and flavanolignan compounds found in milk thistle seeds: (I) Silybinin; (II) isosilybinin; (III) silychristin; (IV) silydianin; (V) taxifolin.

Silymarin has been used to prevent and treat a variety of liver diseases and to protect the liver against toxicity. Double-blind studies of 57 patients with acute hepatitis showed that therapy with silymarin deceased complications, hastened recovery, and shortened hospitalization *(2)*. Silymarin has also proved to be effective in treating chronic hepatitis and cirrhosis *(1,3)*, and more recent studies have shown that silymarin has potential in treating many other human diseases such as skin cancer, prostate cancer, and damaged kidneys *(4–8)*. Milk thistle extracts containing silymarin as the active ingredient have become increasingly popular worldwide. In 2001, sales of milk thistle in the United States increased 13% and now exceed $7 million *(9)*. Annual sales of milk thistle extract in Germany exceed $180 million *(2)*.

Although organic solvents such as hexane, methanol, and ethanol are traditionally used in extracting compounds from plants, water has

recently received special attention as an extraction solvent owing to its "greenness" and comparatively low purchase and disposal costs. Water has been reported to solubilize environmentally aggressive compounds at increased temperatures using elevated pressure to keep the water in the liquid state *(10)*. The dielectric constant, surface tension, and viscosity of water are so dependent on temperature *(10,11)* that with a mere adjustment of temperature and pressure, water can be made to have properties approaching those of organic solvents. Similarly, temperature adjustment enables water to extract both polar and nonpolar compounds from plant matrices. Alvarez Barreto et al. *(12)* studied the batch extraction of silymarin from ground milk thistle seeds using water at 50–100°C. Their results showed that higher yields were obtained at increased temperature, and that the more polar compounds (taxifolin and silychristin) were preferentially extracted at 85°C, while the less polar silybinin was favored at 100°C. Unlike organic solvents, which require defatting of the milk thistle prior to extraction, water extraction did not require defatting.

The present study focused on using hot water as the extraction solvent at 100–140°C to extract silymarin compounds from milk thistle seed meal without prior defatting in a batch dynamic mode. A laboratory apparatus was constructed and used to obtain silymarin compound concentrations as a function of time. Because compound degradation is possible at elevated temperatures, degradation kinetics were also investigated at 140°C using commercial preparations of silymarin.

Materials and Methods

Milk Thistle Seeds and Compounds

Milk thistle seeds were purchased from Frontier Herbs (Norway, IA). Water for extraction was treated in a Milli-Q TM Water System from Millipore (Bedford, MA), and high-performance liquid chromatography (HPLC)–grade methanol was obtained from Honeywell (Muskegon, MI). Silymarin (also known as silybin) was purchased from Sigma (St. Louis, MO) and used in compound degradation studies. Taxifolin standard was obtained from Extrasynthese (Lyon, France). Among the silymarin compound standards, silybinin was obtained from Sigma, and silychristin from PhytoLab (Hamburg, Germany). No standard was available for isosilybinin.

Extraction Apparatus

A hot water dynamic extraction device was constructed using the solubilization apparatus of Miller and Hawthorne *(10)* as a model and was used for pressurized extraction of milk thistle seed meal. Figure 2 shows a schematic diagram of the apparatus. Water, the extraction solvent, was pressurized and pumped using a Bio-Rad 2800 HPLC solvent delivery system (Hercules, CA) to an extraction cell housed in the oven of a Hewlett-Packard 5890 gas chromatography (GC) oven (Wilmington, DE). Before

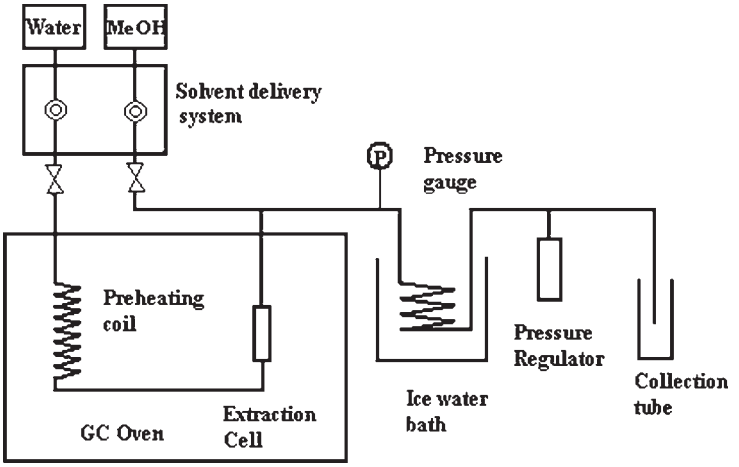

Fig. 2. Diagram of pressurized hot/liquid water extraction apparatus.

entering the extraction cell, the water was fed through a 3-m stainless steel (1.6-mm id) preheating coil. The preheating coil is necessary to ensure that water is at the required operating temperature prior to entering the extraction cell. The stainless steel extraction cell (6.4-mm id, 9.5-mm od, 67.1-mm length) held the ground milk thistle seed meal, along with a layer of washed sea sand, placed just before the cell outlet to prevent seed meal solids from clogging the system. To prevent deposition of the extracted compounds on leaving the oven, methanol was added to the aqueous solution using a Bio-Rad HPLC solvent delivery system through a tee, placed in the oven between the cell and the pressure gage. The pressure gage indicated the pressure under which the extraction is carried out. A 1-m cooling coil, immersed in ice water, was used to cool the water/extracts/methanol mixture to room temperature prior to collection. The mixture was collected in tubes as a function of time and stored for subsequent analysis.

Extraction Procedures

To prepare the solids for extraction, an aliquot of milk thistle seeds was ground to an average particle size of 0.4 mm using a Braun KSM 2B coffee grinder. Approximately 0.5 g of the milk thistle seed meal was packed into the extraction cell along with about 2 g of washed sea sand (40–50 mesh) at the extraction cell outlet to prevent plugging of the cell frit. After the loaded extraction cell was installed in the GC oven, water was pumped through the cell at a constant flow rate via the preheating coil. When water was observed at the collection exit, methanol flow was initiated. The pressure regulator was adjusted to keep the pressure above the saturation pressure of water at the desired temperature selected in the GC oven. When the desired temperature was reached, continuous sample

collection began using preset time intervals for each sample tube. After collection, 1 mL of each sample was evaporated to dryness in a self-constructed evaporator while sparging with compressed nitrogen. The solids were redissolved in 1 mL of methanol, filtered (0.45-µm filter), and analyzed by HPLC.

Compound Degradation Procedures

Silymarin compound degradation studies were carried out at 140°C in stainless steel tubes (2.0-mm id, 3.2-mm od, 152-mm length) completely filled with the silymarin solution so as to exclude air and sealed with end caps on both sides. pH was not monitored in these initial experiments aimed at determining whether degradation was indeed likely present. At the beginning of a degradation experiment, all of the tubes were placed in the GC oven at the desired temperature. Six tubes were removed simultaneously every 10 min and quickly placed in ice water. The liquid was collected and analyzed by HPLC as described below.

Analytical Methods

The concentrations of the individual silymarin compounds and taxifolin were determined by HPLC using a Waters system (Milford, MA) composed of an Alliance 2690 separations module and a 996 Photodiode Array detector controlled with Millennium[32] chromatography software. The HPLC procedure was previously described by Wallace et al. *(13)*. Calibration curves were prepared from standard solutions of taxifolin, silychristin and silymarin. The silybinin standard from Sigma contained two distinct peaks, which are further referred to as silybinin A (the first peak) and silybinin B (the second peak).

Results and Discussion

Extraction

Milk thistle seed extraction experiments were carried out with hot water at 100, 120, and 140°C using the same water flow rate (0.30 mL/min) and seed meal particle size (0.4 mm). The pressures employed in the experiments at these temperatures were approx 1, 4, and 5 atm, respectively. Figure 3 shows results from typical runs at 100, 120, and 140°C, where the compound concentrations from the collected water (methanol added for solubilization is subtracted out) in each sample aliquot from the continuous flow apparatus are plotted as a function of time. Thus, the concentrations presented represent the average concentrations of the four main extracted compounds in an aliquot. As noted in Fig. 3, the concentrations of each of the compounds reached a maximum after a few minutes of extraction time and then fell exponentially with time as the extracted material was removed from the solid sample. The time for obtaining the maximum compound concentration decreased with temperature.

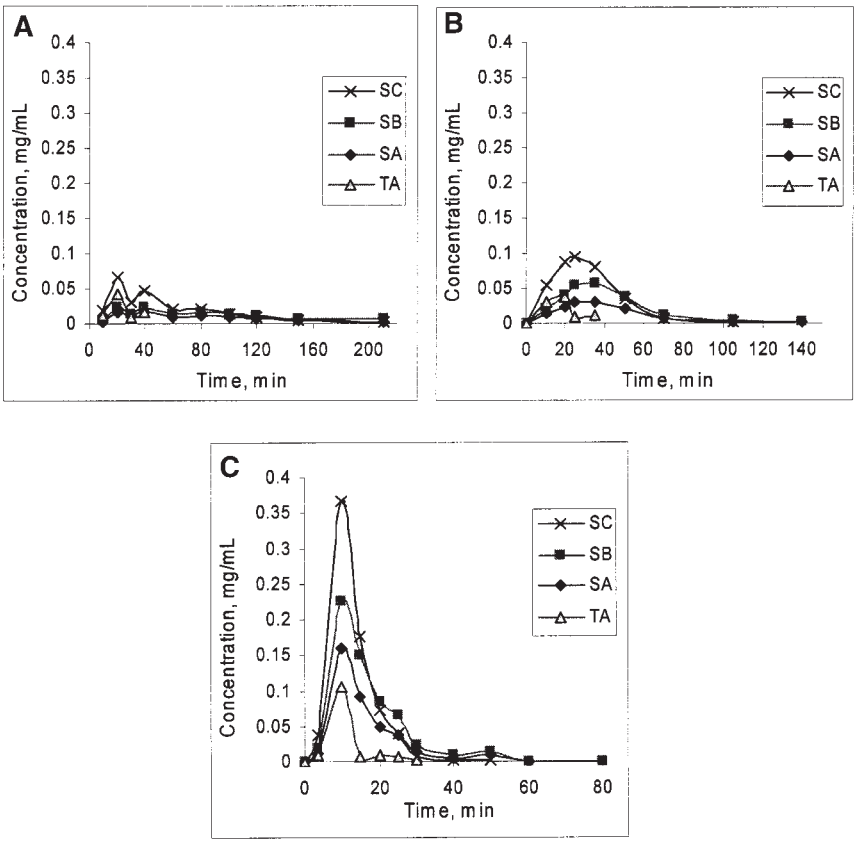

Fig. 3. Concentrations of silymarin compounds and taxifolin at outlet of extraction cell as function of time with extraction temperature at **(A)** 100°C, **(B)** 120°C, and **(C)** 140°C. SC, Silychristin; SB, silybinin B; SA, silybinin A; TA, taxifolin).

The data in Fig. 3 were used to prepare cumulative yield plots as a function of time and each extraction temperature, as noted in Fig. 4. The cumulative yield is defined as the cumulative quantity of extracted compound per mass of seed. These plots are useful in comparing the quantities of each of the compounds extracted but are limited in that they do not show any information about compound degradation. In analyzing the results in Fig. 4, a short, rapid extraction period at the beginning of the extraction was followed by a longer, slow extraction period, shown as a leveling off of the compound yields. Each of the curves for the extracted compounds presented similar shapes at the three different temperatures, reaching essentially the same yield maxima regardless of the experimental condition. This observation was unexpected, since elevated temperatures should result in the extraction of more nonpolar compounds (silybinin A and B), while lower temperatures should favor the extraction of more polar compounds (such as silychristin). In fact, Alvarez Baretto et al. *(12)* saw this preferential extraction effect in studying the extraction

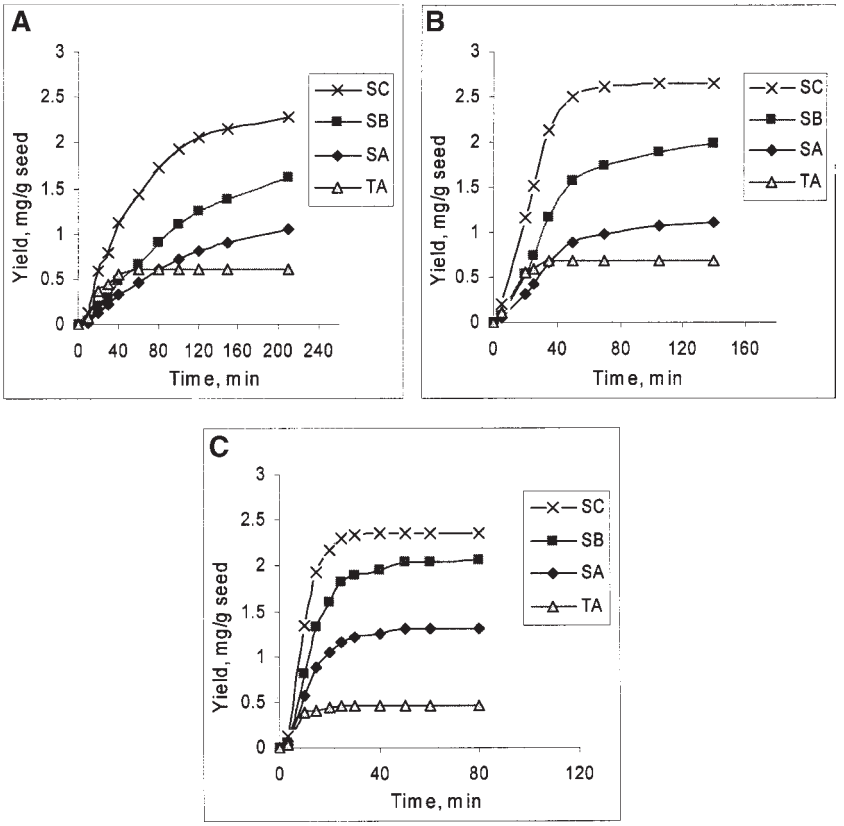

Fig. 4. Extraction yields of silymarin compounds and taxifolin as function of time at **(A)** 100°C, **(B)**120°C, and **(C)**140°C. SC, Silychristin; SB, silybinin B; SA, silybinin A; TA, taxifolin.

of silymarin compounds and taxifolin over a temperature range of 50–100°C. As expected, the maxima were reached faster as the temperature was increased and pseudo equilibrium was approached.

Further comparisons of the extraction data at the three temperatures are shown in Tables 1 and 2. As noted in Table 1, the maximum yields of the silymarin compounds at the three extraction temperatures essentially did not vary, with the yields of silymarin compounds at 120°C being just slightly higher than those at 100 and 140°C. It is known that extraction efficiency increases with temperature. Richter et al. *(11)* theorized that this is owing to enhanced solubilization, higher diffusivity of solutes, and disruption of the strong solute-matrix interaction caused by Van der Waals forces, hydrogen bonding, and dipole forces of solute molecules and active sites on the matrix at increased temperature. According to these theories, the yields of the silymarin compounds and taxifolin should have increased with temperature. Thus, temperature had a positive effect on extraction yield, however, it was perhaps circumvented by a temperature-induced degradation.

Table 1
Yields of Silymarin Compounds and Taxifolin at 100, 120, and 140°C

	Maximum yield (mg/g seed)			
Temperature (°C)	Taxifolin	Silychristin	Silybinin A	Silybinin B
100	0.6	2.3	1.0	1.6
120	0.7	2.5	1.2	2.2
140	0.5	2.4	1.2	2.0

Table 2
Times for Yields of Silymarin Compounds and Taxifolin
to Reach Their Maximum Concentrations at 100, 120 and 140°C

	Time to reach maximum yield (min)			
Temperature (°C)	Taxifolin	Silychristin	Silybinin A	Silybinin B
100	60	200+	200+	200+
120	40	140+	140+	140+
140	30	55	55	55

Wallace et al. *(13)* observed potential thermal degradation of silymarin compounds when extracting milk thistle seed meal with ethanol in a Soxhlet apparatus at 78°C, they noted that the yield of silymarin decreased at long extraction times and that the yields were higher at 60°C. The times for the yields of the silymarin compounds to reach a maximum, shown in Table 2, significantly decreased with increasing temperature. For example, the time for the yield of silychristin to reach its maximum at 140°C was about one-fourth the time at 100°C, with close yields at both temperatures.

Compound Degradation

Preliminary compound degradation studies were performed with solutions of the silymarin compounds, and thus the compounds were unprotected by the plant matrix during heating. The results of the experiment, performed at 140°C, confirm the existence of significant compound degradation. More extensive degradation studies (additional temperatures, degradation product identification) will be performed later. Exponential decay of silybinin A and silybinin B was observed, as demonstrated in Fig. 5. After 10 min of incubation of the silymarin solution at 140°C, no silychristin was detected by HPLC.

Thermal degradation can be modeled as a first-order irreversible reaction, which can be described by Eq. 1:

$$\frac{dC_C}{dt} = -kC_C \tag{1}$$

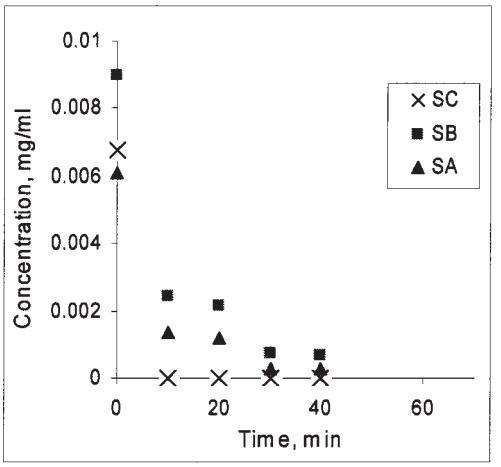

Fig. 5. Concentrations of silybinin A, silybinin B and taxifolin at 140°C as function of time during compound degradation studies. SC, Silychristin; SB, silybinin B; SA, silybinin A.

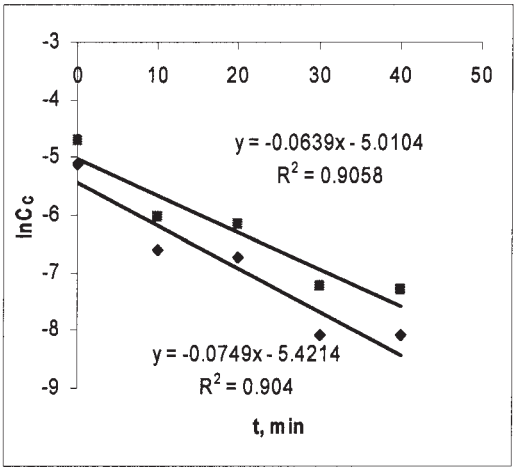

Fig. 6. Degradation kinetics for silybinin A (◆) and silybinin B (■) at 140°C as function of time.

where C_C is the concentration of the compound (g/L), t is the reaction time (min), and k is degradation reaction constant (min^{-1}). The degradation constants obtained for silybinin A and silybinin B at 140°C were 0.064 and 0.075 min^{-1}, respectively, as shown in Fig. 6. The degradation constant for silychristin is at least an order of magnitude higher, assuming nearly complete conversion in 10 min. Thus, the yields of compounds in extracting compounds at elevated temperatures are indeed compromised by compound degradation.

Conclusion

The extraction of silymarin compounds and taxifolin from milk thistle seed meal using hot water at 100–140°C was investigated. The maximum extraction yield of each of the silymarin compounds did not increase with temperature, most likely because compound degradation occurred. In addition, preferential extraction of nonpolar compounds over polar compounds was not observed at elevated temperature, as previously observed by Alvarez Baretto et al. *(12)*. However, the time required for the yields of the compounds to reach their maxima was reduced from 200 to 55 min when the extraction temperature was increased from 100 to 140°C. Significant degradation of unprotected (cell matrix not present to slow degradation) silymarin compounds was observed and first-order degradation kinetics were obtained at 140°C. Future work needs to focus on obtaining true equilibrium data as well as additional extraction and degradation data.

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